Improving yield and economic viability of peanut production in Papua New Guinea and Australia

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Procedures and protocols to maintain purity and viability of peanut (groundnut) germplasm

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Abstract

Successful genebank operations should ensure that pure and healthy seeds are maintained for utilisation in crop improvement and conserving for posterity. Maintaining peanut germplasm in the genebank requires considerable attention. Some protocols followed to maintain purity and viability of peanut germplasm are outlined in this paper. Some distinct measures required for maintenance of peanut germplasm are: (i) raising the peanut crop in well drained light soils, (ii) growing a minimum of 160 plants per accession to retain genetic integrity, (iii) verification of accessions' identity at various stages of crop growth to remove off-type and diseased plants, (iv) harvesting crop at optimum maturity to obtain healthy seeds, (v) avoiding exposure of the freshly harvested pods to strong sunlight (temperature >40°C) to maintain seed viability in storage, (vi) avoiding damage while picking and shelling of pods manually, (vii) conserving seeds at appropriate moisture content, in medium term (4°C; 30% RH) at 7% and in long term (-20°C) at 3–5% and (viii) monitoring of seed stock, viability and health at regular intervals to assess the need for regeneration.

As part of the Australian Centre for International Agricultural Research (ACIAR) project ASEM 2001/055, ICRISAT supplied 46 peanut (groundnut) breeding lines for evaluation in Papua New Guinea (PNG) environments to identify varieties with superior yield and quality over the local check which could be introduced for cultivation in PNG. It is critical that the PNG organisations take responsibility for maintaining genetic purity of the newly introduced peanut germplasm. This paper describes the procedures and protocols that are followed at ICRISAT for maintaining the genetic purity of peanut germplasm. These lines can be maintained by following the procedures described in this paper.

Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is the third largest oilseed crop after soybean and seed cotton, globally. Peanut was cultivated on more than 24 m ha annually during the 2002–04 triennium, producing more than 35 Mt and with productivity of about 1437 kg ha^{-1} peanut-in-shell (FAO 2002–04). As well as producing edible oils, peanut seeds are rich in protein. About two-thirds of world peanut production is used to extract oil and the remainder is in the form of protein-rich, edible products. Peanut is native to southern Bolivia and north-western Argentina. Sixty-nine species are known to occur in the genus *Arachis*, which have been classified into nine sections. Peanut is a tetraploid ($2n = 40$) while some wild species are diploid too. It is a highly self-pollinated crop. In many countries, peanut is grown by smallholder farmers as a cash crop. The value of genetically pure and viable germplasm seed for use in crop improvement is well recognised.

The genebank of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) holds 14,966 accessions of cultivated *Arachis* spp. and about 453 of wild *Arachis*. The advanced/elite breeding lines and/or germplasm lines are supplied to needy countries by ICRISAT under appropriate agreement (Material Transfer Agreement) for evaluation and release to peanut growers. During 2001–02, as a part of the ACIAR project ASEM 2001/055, ICRISAT supplied 46 (20 short-duration; 26 medium-duration) elite breeding lines (Table 1) for evaluation under PNG environments and assisted the project in developing and implementing a technical work plan for the varietal trials. It is expected that some of the promising varieties will be eventually released for cultivation by smallholders in PNG. However, it is important that genetic purity and viability of the germplasm is

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Table 1. List of groundnut varieties supplied by ICRISAT to Papua New Guinea during 2001–2002

Serial no.	Short-duration genotypes	Serial no.	Medium-duration genotypes
1	ICGV 94299	21	ICGV 92029
2	ICGV 94341	22	ICGV 94016
3	ICGV 94350	23	ICGV 94037
4	ICGV 94357	24	ICGV 94040
5	ICGV 94358	25	ICGV 92160
6	ICGV 94361	26	ICGV 93058
7	ICGV 95244	27	ICGV 93115
8	ICGV 95245	28	ICGV 93123
9	ICGV 95248	29	ICGV 93139
10	ICGV 95271	30	ICGV 93143
11	ICGV 95278	31	ICGV 94043
12	ICGV 95290	32	ICGV 94049
13	ICGV 95299	33	ICGV 94113
14	ICGV 95319	34	ICGV 94215
15	ICGV 95322	35	ICGV 95163
16	ICGV 95256	36	ICGV 95165
17	ICGV 96468	37	ICGV 95172
18	ICGV 96469	38	ICGV 95179
19	ICGV 96470	39	ICGV 96066
20	ICGV 96466	40	ICGV 96073
		41	ICGV 96081
		42	ICGV 96100
		43	ICGV 96107
		44	ICGV 96108
		45	ICGV 96110
		46	ICGV 96234

maintained as a separate exercise by the PNG agricultural R&D agencies.

In this paper, we have attempted to explain the various aspects of purity and viability of peanut germplasm seeds and protocols to maintain them. The concern for genetic purity and viability is of paramount importance in genebank operations and appropriate procedures need to be outlined (Rao and Bramel 2000).

Germplasm purity can be identified at two levels: physical purity and genetic purity.

Physical purity

Germplasm accessions should be free from seeds of other crops, weeds, plant debris or soil. This can be ensured easily with good crop management, and pre- and post-harvest care.

Genetic purity

Genetic purity means that the germplasm accessions should be maintained in close to the same structure (genetic composition/entities) as they were originally secured from the source. Maintaining such structure will also need attention in subsequent germplasm handling, namely at the time of regeneration, characterisation, labeling, seed cleaning and processing.

Protocol for maintaining genetic purity in peanut germplasm

Peanut descriptors

A set of traits that are important for diagnostic purposes, agronomic characterisation and grain nutritional value are recognised and known as 'descriptors' of the crop species. The 'peanut descriptors' (IBPGR and ICRISAT 1992) were developed jointly by ICRISAT and International Plant Genetic Resources Institute (IPGRI) researchers together with other internationally known scientists. Each peanut germplasm accession in the ICRISAT genebank has been characterised for these traits and data are maintained on the ICRISAT website (www.icrisat.org) for the benefit of users. Some diagnostic traits of peanut that could be used for verifying genetic purity of germplasm accessions are given below:

Growth habit Plant growth patterns can be grouped into the following seven classes: procumbent-1, procumbent-2, decumbent-1, decumbent-2, procumbent-3, erect and other (Figure 1), which are recorded at the pod-setting stage.

Plant height (cm) The height of the main axis is measured on 10 representative plants from ground until terminal bud at 60–85 days after emergence.

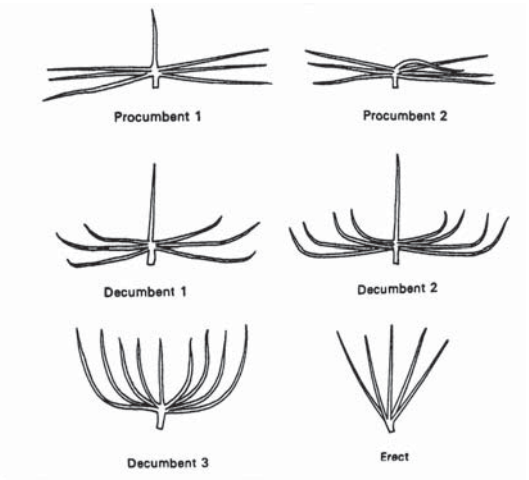

Figure 1. Growth habit classes in peanut germplasm (Source: Rao and Bramel 2000)

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Stem pigmentation Presence of anthocyanin pigmentation on the stem is recorded at flowering stage as either present or absent.

Stem hairiness Hairiness on the main stem of peanut germplasm is recorded at flowering stage as one of the following five classes: (i) glabrous (no hairs); (ii) sub-glabrous (hairs in one or two rows along main stem); (iii) moderately hairy (three or four rows along the main axis); (iv) very hairy (most of the stem surface covered with hairs); and (v) woolly (most of the stem surface covered with long hairs).

Branching pattern The pattern of branching is recorded in the following five classes: (i) alternate, (ii) sequential, (iii) irregular with flowers on main stem, (iv) irregular without flowers on main stem and (v) others (Figure 2).

Leaflet length (cm) The length of the apical leaflet of the fully expanded third leaf on the main stem is recorded on 10 leaflets from different plants.

Leaflet width (cm) The width of the apical leaflet of the fully expanded third leaf on the main stem is recorded at its widest portion on 10 leaflets from different plants.

Leaflet shape The shape of fully expanded apical leaflet of the third leaf on the main stem is recorded in 14 classes (Figure 3).

Days to 50% flowering Number of days from emergence to the day on which 50% plants of an accession have flowered.

Flower colour Seven flower colours could be identified in peanut. The colour of the front face of the vexillum excluding the streak portion of the fresh and fully opened flowers is recorded. The seven colours are: white, lemon, yellow, orange-yellow, orange, dark orange and garnet/brick red.

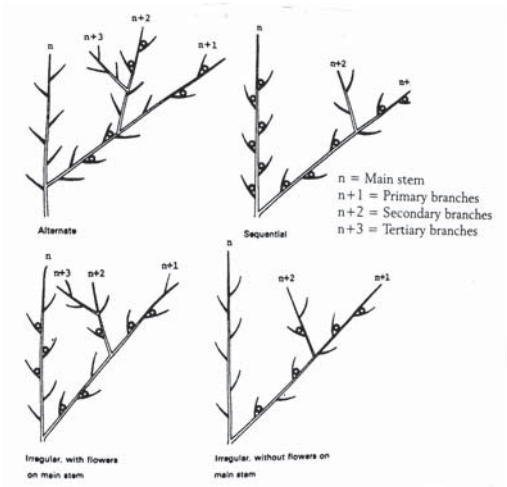

Figure 2. Branching pattern classes in peanut germplasm (Source: Rao and Bramel 2000).

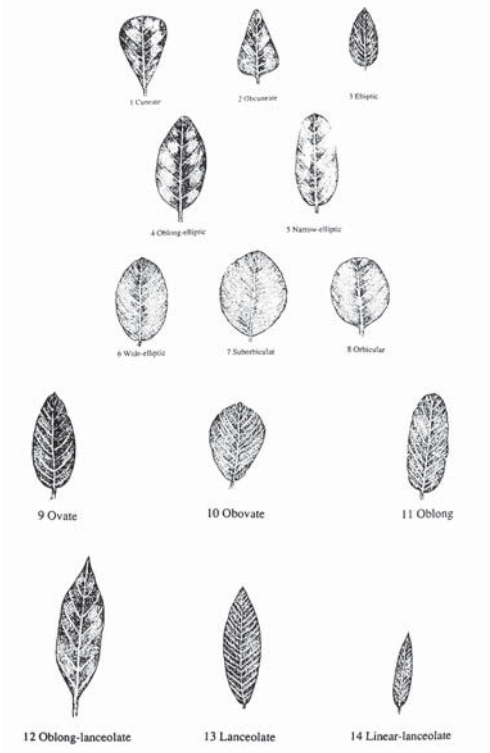

Figure 3. Leaflet shape classes in peanut germplasm (Source: Rao and Bramel 2000)

Pod beak Pod beak (i.e. tip of the indehiscent pod) is recorded in five classes: absent, slight, moderate, prominent and very prominent (Figure 4).

Pod constriction The degree of pod constriction is recorded in five classes: no constriction, slight, moderate, deep, and very deep constriction (Figure 5).

Pod reticulation Also known as pod venation, this characteristic is recorded in five classes: no reticulation, slight, moderate, prominent, and very prominent.

Pod length (cm) This is the average of 10 representative mature pods.

Pod width (cm) This is the average of 10 representative mature pods.

Seed number per pod The pattern of seed number in pods is an important diagnostic and agronomic trait. The number can vary from 1–4, rarely 5 (Table 2). The first number indicates most frequent number of seeds in pods of an accession, the second indicates second most frequent number and so on.

Primary seed colour This should be recorded within 1 month of harvest on dry and mature seeds. Twenty primary seed colors can be found in peanut germplasm accessions (Table 3).

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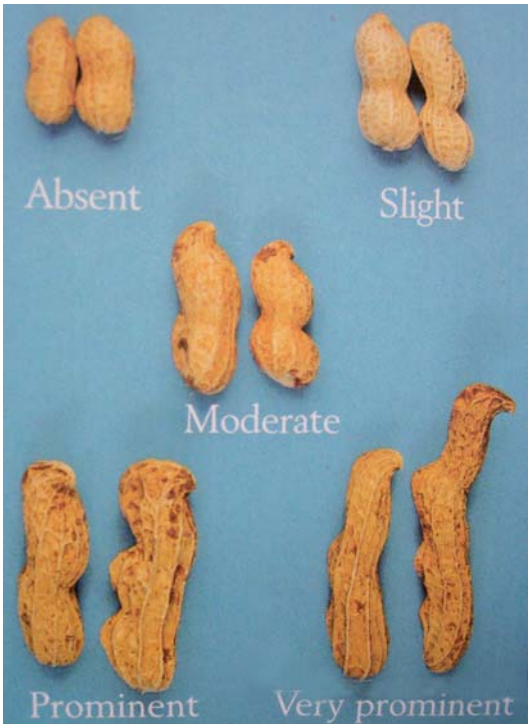

Figure 4. Pod beak classes in peanut germplasm (Source: Nigam et al. 2004)

Table 2. Commonly found seed numbers per pod in groundnut germplasm

1.	2-1
2.	2-3-1/2-1-3
3.	3-2-1/3-1-2
4.	2-3-4-1/2-4-3-1/2-3-1-4/2-4-1-3/2-1-3-4/2/1/4/3
5.	3-2-4-1/3-2-1-4
6.	3-4-2-1/3-4-1-2
7.	4-3-2-1/4-2-3-1
8.	4-3-1-2/4-2-1-3
9.	3- or 4-seeded with occasional 5-seeded pods

Table 3. Major colours found on peanut germplasm seeds

1	White	8	Dark tan	15	Light purple
2	Off-white	9	Greyed orange	16	Purple
3	Yellow	10	Rose	17	Purple
4	Very pale tan	11	Salmon	18	Dark purple
5	Pale tan	12	Light red	19	Very dark purple
6	Light tan	13	Dark red	20	Other
7	Tan	14	Purplish red		

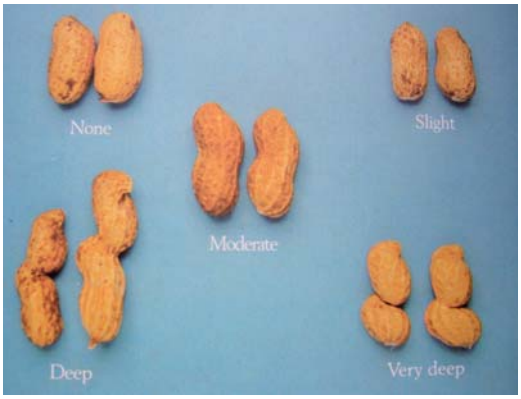

Figure 5. Pod constriction classes in peanut germplasm (Source: Nigam et al. 2004)

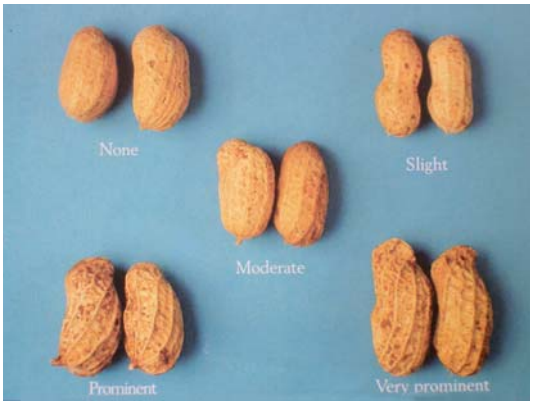

Figure 6. Pod reticulation classes in peanut germplasm (Source: Nigam et al. 2004)

Protocols for peanut germplasm regeneration to ensure optimum genetic makeup and healthy seeds

Sowing

To ensure optimum genetic structure of the accessions, a minimum of about 160 plants per accession are grown for regeneration. A plant-to-plant spacing of 60 × 10 cm will be adequate for the accessions of subsp. *fastigiata* and 60 × 15 cm for subsp. *hypogaea* so that each plant could be examined conveniently. Seeds of subsp. *hypogaea* should be treated with Ethrel® (2-chloroethylphosphonic acid, 39%) solution (3 mL L^{-1}) to break seed dormancy and ensure

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even germination. The field selected for regeneration should be well drained with light textured soil and a good calcium status. The field should have a minimum gap of 2 seasons between two peanut crops. This is to overcome the problem of peanut volunteers from the previous crop and also to avoid disease and insect buildup in the soils. The crop could be provided with 60 kg P_2O_5 ha^{-1} as basal fertiliser, and 60 kg calcium (400 kg gypsum) ha^{-1} at 40 days after sowing.

Cultural practices

To ensure adequate seed yield, the crop should be kept weed, insect and disease free. The crop should be maintained with adequate soil moisture to produce healthy seeds by irrigating the field when necessary. The crop should be inspected at various stages to verify the genetic identity and health of the accessions and rogue out the doubtful/ diseased plants.

Harvesting

Determining the right harvesting time in peanut is crucial. As an indeterminate plant, peanut can produce flowers until maturity. This behaviour results in fruits being at different stages of development, making it difficult to decide the harvesting date. To judge maturity, various parameters have been designed, such as optical density of oil (Sharon 1963; Young and Holley 1965), arginine maturity index (Hammons et al. 1978), methanol extract (Holiday et al. 1976, 1979; Pearsson et al. 1973), kernel density (Aristizabal et al. 1969), internal pericarp colour (Gilman and Smith 1977), seed hull maturity index (Pattee et al. 1974), maturity protein marker (Basha 1990), cumulative thermal time (Leong and Ong 1983; Mohamed 1984; Williams et al. 1975). These parameters are too technical and not easy to practise. Each one of them has some shortcomings. In simple words, however, optimum harvesting time is indicated by (i) when about 75% pods have matured, (ii) leaves start yellowing and old leaves start shedding, (iii) pods becoming hard and tough with dark tannin coloration inside shell, (iv) kernel surface becoming smooth and (v) testa developing seed colour characteristics of the genotype.

Harvesting is done by uprooting the plants and picking the pods manually. Pods of Spanish, Valencia, and Virginia Bunch types are confined to the base of plant and pulling plants from soil brings out most of the pods. In the Virginia-runner type, however, pod formation takes place all along the creeping branches. Therefore, plants are pulled out with the help of shovel or spade.

If soil is hard, lightly irrigate the crop 1 day before harvesting.

Leave the uprooted plants in the field with pods turned up for 1–2 days for drying and then pluck the pods.

When the day temperature reaches higher than 40°C during harvesting, dry the uprooted plants under shade or pick the pods immediately. If the highly moist, fresh pods (30–40% moisture) are exposed to strong sunlight (temperature >40°C) even for 1 day, the seed viability is affected.

After drying the pods in shade for 3–4 weeks, shell them manually, process, and pack the seeds for conservation.

Protocols for efficient germplasm seed conservation

Conserving germplasm as seed in contrast to pods is convenient, scientific, and cost effective. The gain in seed longevity is very marginal while conserving peanut in-shell. In the medium-term conservation (MTC) facility, germplasm conservation as seed was about three times more cost effective than as peanut-in-shell (Rao et al. 2000). This is primarily due to the larger space occupied by peanut-in-shell.

After shelling, let the seeds dry in shade or in a controlled environment to moisture content about 7% for MTC (4°C, 30% RH) and 3–5% for long-term conservation (LTC) (-20°C).

It is desirable to pack the seeds in air-tight, screwed lid containers for MTC (active collection) and in non-permeable, vacuum sealed containers for LTC to ensure longer viability.

Ensuring optimum viability of the germplasm seed is important. Viability refers to the ability of seed to germinate and produce a healthy plant. Before the germplasm is taken to LTC, seed germination is recorded. Depending on the initial germination per cent, plans are made for subsequent germination tests. For active collection, tests are made after 8 years on the accessions that had >95% germination at initial stage, after 5 years on accessions having 85–95% germination and after 3 years on accessions that had <85% germination. For base collection, viability should be monitored after 10 years on accessions that had >95% at the initial stage, after 8 years on accessions having 85–95% germination and after 5 years on accessions that had <85% germination. Seeds are also examined for the seed associated micro-organisms and assessment is made for possible damage. If the damage is conspicuous, seeds will be treated with an appropriate chemical before the next regeneration to ensure production of healthy seeds.

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Protocol for handling wild *Arachis* germplasm

Wild *Arachis* spp. require additional care to maintain their germplasm resources. Some of the species require longer duration to set seeds, and some produce very few, small sized, and distantly placed pods. Some species do not set pods and seeds and are maintained vegetatively. Wild *Arachis* species are better maintained in controlled environmental facilities, namely in glasshouse where plants are maintained year round. Alternatively, plants are raised in pots placed in isolated and protected area. The following are some guidelines to maintain the wild *Arachis* germplasm.

Use big size earthen or plastic pots (40 × 30 cm) or concrete rings (65 × 85 cm). Cover the hole at the bottom of the pot with pieces of rubble. Fill the container with pasteurised soil mixture containing three parts red soil, two parts sand and one part farmyard manure. Seeds should be dressed with appropriate fungicide and sown at a depth of 3–4 cm. Many of the wild *Arachis* species have seed dormancy so 2–3 drops of Ethrel® (3 mL L^{-1}) should be applied to seeds before they are covered with soil. Take care to ensure adequate soil moisture in pots and apply gypsum at 10 g per pot 50 days after sowing.

Maintenance of species that are vegetatively propagated

- Use a 20 cm long rhizome cut from the mother plant.
- Soak the rhizomes in Bavistin® suspension (3 g L^{-1}) for 5 minutes.
- Plant the rhizomes in a potting mixture consisting of three parts red soil, two parts sand and one part farmyard manure.
- Plant the rhizomes 5 cm deep, preferably in plastic or earthen pots.
- Maintain the rhizomes in a glasshouse at 25 ± 2°C until they are established.
- It helps to maintain the rhizomes under alternating dry and wet conditions until they are established.
- Rhizomes require 1 month for establishment after which they can be transferred to the field.

Conclusion

Present and future crop improvement programs require genetically pure and healthy germplasm material. Pure and healthy germplasm in genebanks provides the genetic bricks for research by the future generations. Therefore, all possible care should be taken for maintaining pure and healthy seeds in the genebanks.

References

Aristizabal L., Burns E.E. and Kunze O.R. 1969. Physical, chemical and organoleptic properties of peanut separated in a controlled air stream. Transactions of American Society of Agricultural Engineers. 12(3), 298–301.

Basha S.M. 1990. Protein as indicator of peanut seed maturity. Journal of Agricultural and Food Chemistry 38: 373-376.

FAO 2002–04. Food and Agriculture Organization of the United Nations. Website www.fao.org

Gilman D.F. and Smith O.D. 1977. Internal pericarp color as a subjective maturity index for peanut breeding. Peanut Science 4, 67–70.

Hammons R.O., Tai P.Y.P. and Young C.T. 1978. Arginine maturity index: Relationship with other traits in peanuts. Peanut Science 5, 68–71.

Holiday C.E., Williams E.J. and Chew V. 1976. An evaluation of two objective methods for estimating maturity in peanut. (Abstr.) Proceedings of the American Peanut Research and Education Association 8, 78.

—1979. A methods for estimating peanut maturity. Journal of Food Science 44, 254–256.

IBPGR and ICRISAT 1992. Descriptors for groundnut. International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Leong S.K. and Ong C.K. 1983. The influence of temperature and soil water deficit on the development and morphology of groundnut. Journal of Experimental Botany 34, 1551–1561.

Mohamed H.A. 1984. Varietal difference in the temperature responses of germination and crop establishment. PhD Thesis, University of Nottingham, UK.

Nigam S.N., Giri D.Y. and Reddy A.G.S. 2004. Groundnut seed production manual. Patancheru 502324, AP, India: International Crops Research Institute for the Semi-Arid Tropics. pp 28.

Pattee H.E., Johns E.B., Singleton J.A. and Sanders T.H. 1974. Composition changes of peanut fruit parts during maturation. Peanut Science 1, 57–62.

Pearson J.L., Holiday C.E., Butler J.L., Williams E.J. and Troeger J.M. 1973. Objective determination of optimum harvest maturity (Abstract). Proceedings of the American Peanut Research and Education Association 5, 197.

Rao N.K. and Bramel P.J. 2000. Manual of genebank operations and procedures. ICRISAT, Technical Manual no 6. pp 190.

Sharon H.D. 1963. Measurement of peanut quality and factors influencing it. Peanut Improvement Working Group Proceedings, Oklahoma State University, pp. 1–15.

Williams J.H., Wilson J.H.H. and Bates G.C. 1975. The growth of groundnut (*Arachis hypogaea* cv. Makulu) at three altitudes in Rhodesia. Rhodesian Journal of Agricultural Research 13, 33–43.

Young C.T. and Holley K.T. 1965. Comparison of peanut varieties in storage and roasting. Georgia Agricultural Experiment Station Technical Bulletin No. 41, p. 30.